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Shedding light on the geographical distribution, population size and inflorescence coloration variety of Royal Irises (*Iris* section *Oncocyclus*) family in West Bank, Palestine*

Johann Gabriel Gedeon[1**], Anton Issa Khalilieh[1**]

1 *Nature Palestine Society, Hanna Naqqara, Ramallah, West Bank, Palestine*

Corresponding author: Anton Issa Khalilieh (anton@naturepalestine.org)

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Abstract

Palestine has rich floristic diversity due to its location, diverse biogeographical zones, and various topographical features. The Royal Irises (*Iris* section *Oncocyclus*), are a monophyletic group of herbaceous geophytes, consisting of 33 species, endemic to the Middle East. Within the West Bank of Palestine, three Royal Irises are documented. However, their distribution, population size, and status are not studied comprehensively. This study focused on evaluating the occurrence, distribution range, population size, status, number of flowers, inflorescence coloration variety, and habitats of the vulnerable and endemic *Iris haynei*, the endangered and sub-endemic *Iris lortetii*, and the vulnerable and sub-endemic *Iris atrofusca*. *I. lortetii* was found in 10 sites within Nablus district, with more than 12625 clones and 25088 flowers flowers recorded. *I. haynei* were documented in 4 sites within Jenin district, three are new to science, where 10729 clones and 9562 flowers were recorded. *I. atrofusca* was reported in 13 localities in Tubas and Ramallah districts, where 1853 clones and 8460 flowers were recorded, whereas six locatities are new to science. 12, 12, and 7 unique variations in coloration of inflorescences were observed for *I. lortetii*, *I. atrofusca*, and *I. haynei*, respectively. The results indicate that the population size and the distribution range of the three Royal Irises are larger than previously reported, whereas the documented inflorescences' coloration variations are unique and considerable. This study sheds light on hotspot occurrence sites of endangered and vulnerable species in Palestine, which can assist in laying adequate conservation actions and management plans in the future.

Keywords

Anthropogenic effects, Conservation, Distribution map, Endangered, Endemic, Geophyte, Iris, Mediterranean basin, Vulnerable

Introduction

Palestine is located in the eastern part of the Mediterranean basin hotspot within the Orontes Valley and Levantine Mountains corridor hotspot (Auerbach and Shmida 1985; Médail and Quézel 1997; Myers et al. 2000; CEPF 2017), at a cross junction between Eurasia and Africa. The West Bank area of Palestine is divided into four major biogeographic zones from west to east, namely, the Mediterranean, the Irano-Turanian, the Saharo-Arabian, and the Sudanian-Ethiopian chorotypes (Zohary 1972; Danin 1991; Soto-Berelov et al. 2015). In addition, Palestine encompasses a unique topography where a short journey of 30-minute drive from central West Bank, one can shift from an altitude of 1016 meters above mean sea level (*mamsl*) to the northern shores of the Dead Sea, at an altitude of 420 meters below sea level (*mbmsl*), the lowest terrestrial elevation on Earth. Moreover, annual precipitation

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** Both authors contributed equally to this work.

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varies significantly among the different biogeographical zones, in general, rainfall decreases orographically from 500–600 mm/year in western slopes to 150–45 mm/year in eastern slopes and latitudinally from 700 mm/year in the north to 80–100 mm/ year in the south (Enzel et al. 2008; Ministry of Agriculture 2008, 2009; UNDP 2010). All these factors influence the richness in floral species in Palestine (Zohary 1973; Zohary 1972; Feinbrun-Dothan 1986).

Despite its relatively small area (5856 km^2), about 1612 vascular plant species have been recorded in the West Bank alone, belonging to 117 families, of which thirteen species (0.81%) are endemic (Shmida 1984; Médail and Quézel 1997; Nevo et al. 1999; Al-Sheikh et al. 2000; Cuttelod et al. 2009; Al-Sheikh 2019; Al Sheikh and Qumsiyeh 2021a; Pahl and Qumsiyeh 2021). However, the flora of Palestine is not well studied, up to now no comprehensive surveys were conducted (Levin and Shmida 2007). Within the last few years, there have been few studies focusing on endemic and rare plant species (Al Sheikh and Qumsiyeh 2021b; Pahl and Qumsiyeh 2021; Qumsiyeh and Al-Sheikh 2023; Gedeon and Qumsiyeh 2023). Determining the geographical distribution range, population size, and status of narrow endemics, provides opportunities for understanding evolutionary processes over small spatial scales, studying the variations among the species, providing information for sampling strategies, reintroduction, translocations, genetic enhancement, and developing management plans for conservation actions and strategies in the future (Woodruff 2001).

Royal Irises (*Iris* section *Oncocyclus*) of the family *Iridaceae* L. (2n = 20 chromosomes) are a monophyletic young group of herbaceous geophytes, consisting of 33 species endemics to the Middle East. They serve as models for the evolutionary processes of speciation and pollination ecology (Sapir et al. 2002; Sapir and Shmida 2002; Saad and Mahy 2009; Lavi and Sapir 2015; Volis et al. 2019; Roguz et al. 2020; Bar-Lev et al. 2021; Lozada-Gobilard et al. 2023). These Irises are characterized by their large flowers (6–15 cm in diameter) and few flowers per stalk (Avishai and Zohary 1977, 1980). The self-incompatible Irises scatter across rocky slopes of drained limy garrigue habitats (Wilson et al. 2016). Each flower has three "pollination tunnels" (Monty et al. 2006; Sapir et al. 2006; Lozada-Gobilard et al. 2023) with marked landing platforms, attracting large invertebrate pollinators. The *Oncocyclus* species are almost isolated from one another geographically, and ecologically, by temporal mechanisms, and preferences to pollinators (Avishai and Zohary 1980; Sapir et al. 2005). However, no evidence of reproductive isolation barriers has been documented or evaluated to elucidate the speciation among the Royal Irises (Avishai and Zohary 1980; Osmolovsky 2022). Seven species of *Iris* genus have been recorded in the West Bank (Zohary 1972; Al-Sheikh 2019), most of them are endangered sub-endemic plants (Sapir et al. 2003). Three of them belong to the Royal Irises, *Iris* section *Oncocyclus* (*Iris haynei* Baker, *Iris lortetii* Barbey, and *Iris atrofusca* Baker), which are covered by this study.

I. lortetii inhabits semi-to-dry rocky garrigue areas in south Lebanon, upper Galilee, and Nablus hills with a limited and scattered distribution range of 220 km^2 (Feinbrun-Dothan 1986; Sapir 2016b; Shemesh et al. 2018). The reported upper elevation limit of the *I. lortetii* is 600 m, while the lowest elevation limit is 150 m (IUCN 2016). The population of *I. lortetii* has been decreasing, which can be attributed to several anthropogenic and environmental threats (Shmida and Pollak 2007; Sapir 2016b; Shemesh et al. 2018). *I. atrofusca* is documented to grow on loess and rendzina soils where annual precipitation is less than 200 mm (Avishai and Zohary 1980). The population of *I. atrofusca*, across its distribution range, is decreasing (Danin 2015). Its distribution range is wider than other royal Irises. It can be found in the north-central parts of the West Bank, the northern-western mountains of Jordan, and the northern area of the Al-Naqab desert (Sapir and Shmida 2002; Sapir et al. 2005; Osmolovsky et al. 2022). The reported upper elevation limit of the *I. atrofusca* is 800 m, while the lowest elevation limit is 0 m (IUCN 2016). The third Royal *Iris* species is *I. haynei*, whose distribution range is restricted to the northeast of the West Bank within a limited geographical range of the Faqqua-Jalbun area and the hillsides of Mount Gilboa (Feinbrun-Dothan 1986; Danin 2015). The reported upper elevation limit of the *I. haynei* is 550 m, while the lowest elevation limit is 0 m (IUCN 2016). The population of the *I. haynei* is decreasing (Danin 2015). *I. haynei* and *I. atrofusca* are two closely related, narrow endemics, distributed vicariously along an ecogeographical north-south gradient in the West Bank. This study aims to update and evaluate the occurrence, distribution range, population size, and the diversity of inflorescence-coloration variances of the *I. lortetii*, *I. atrofusca*, and *I. haynei*, in the north-central part of the West Bank of Palestine.

Materials and methods

a) Study area

An extensive Royal Irises survey was conducted in the north-central region within Nablus, Tubas, Jenin, and Ramallah districts, where more than 355 km^2 (355000 dunam; 1 dunam = 0.1 hectare = 0.001 km^2) were explored. The focus of the study was on areas previously identified within the potential geographical distribution range of the *I. haynei*, *I. lortetii*, and *I. atrofusca* (Fig. 1, Feinbrun-Dothan 1986; Danin 2015; Horvitz 2016; Osmolovsky et al. 2022). Site selection was based on an examination of available literature (peer-reviewed and gray), previous observations by Nature Palestine Society's (NPS) team, observations made by members of local communities and posted on social media, and via interviewing local community members (e.g., farmers, shepherds) for potential sites. The study areas of the *I. lortetii*, *I. atrofusca*, and *I. haynei* comprise 219 km^2, 128 km^2, and 9.45 km^2, respectively. In addition, several local sites were examined arbitrarily (see results section for more details), especially within the Faqqua-Jalaboun area in the northeastern part of the West Bank, where the distribution range of the *I.*

haynei population is known to occur (Feinbrun-Dothan 1986; Danin 2015; Horvitz 2016).

b) Field survey and data collection

A comprehensive active survey was conducted during the spring seasons of the years 2020 and 2021 for *I. atrofusca*, while for the *I. lortetii* and *I. haynei*, the survey was carried out during the spring season of 2023. All field surveys were conducted during the flowering period of the three Iris species that extend between the end of February and April (Feinbrun-Dothan 1986; Danin 2015). In addition, records were taken for other threatened, rare, sub-endemic, or endemic plant species when observed. Rare is a plant taxon which is infrequently encountered, if its distribution fits one of the three criteria of narrow or highly disjunctive geographical range, high habitat specificity, or consistently low population size (Rabinowitz 1981; Longton and Hedderson 2000).

Endemism was applied to those taxa e.g. *I. haynei* whose area of distribution is restricted to one biogeographical unit (Palestine) (see discussion in Breman et al. 2020; Feinbrun- Dothan 1986; Danin 2015; Hurdu 2012). *I. lortetii* and *I. atrofusca* have been considered sub-endemics since more than 70% of their total population is within the studied area (Palestine), with the remaining population located within the surrounding Levant region (Lebanon, Syria, Jordan)—for instance, within South Lebanon in the case of *I. lortetii* (Feinbrun-Dothan 1986; Danin 2015; Hurdu et al. 2022). The terms endemic and subendemic are used for range-restricted species (Kruckeberg and Rabinowitz 1985).

The specific habitat and some of its distinct vegetation within each explored site holding the royal Irises was examined and described. To cover the largest possible area within the shortest time, binoculars (Swarovski EL 8.5*42, Swarovski Optik, Absam, Tyrol, Austria) and telescopes (Swarovski 65 mm, 20–60×, Swarovski Optik, Absam, Tyrol, Austria) were used to explore potential sites from a distance (200 m – 1 km) to locate the species. When a clone (fan-like cluster of leaves; for instance, *I. lortetii* has a fan-like cluster of 6–8 leaves) or a clump of Royal Irises were observed, the site was actively searched. Moreover,

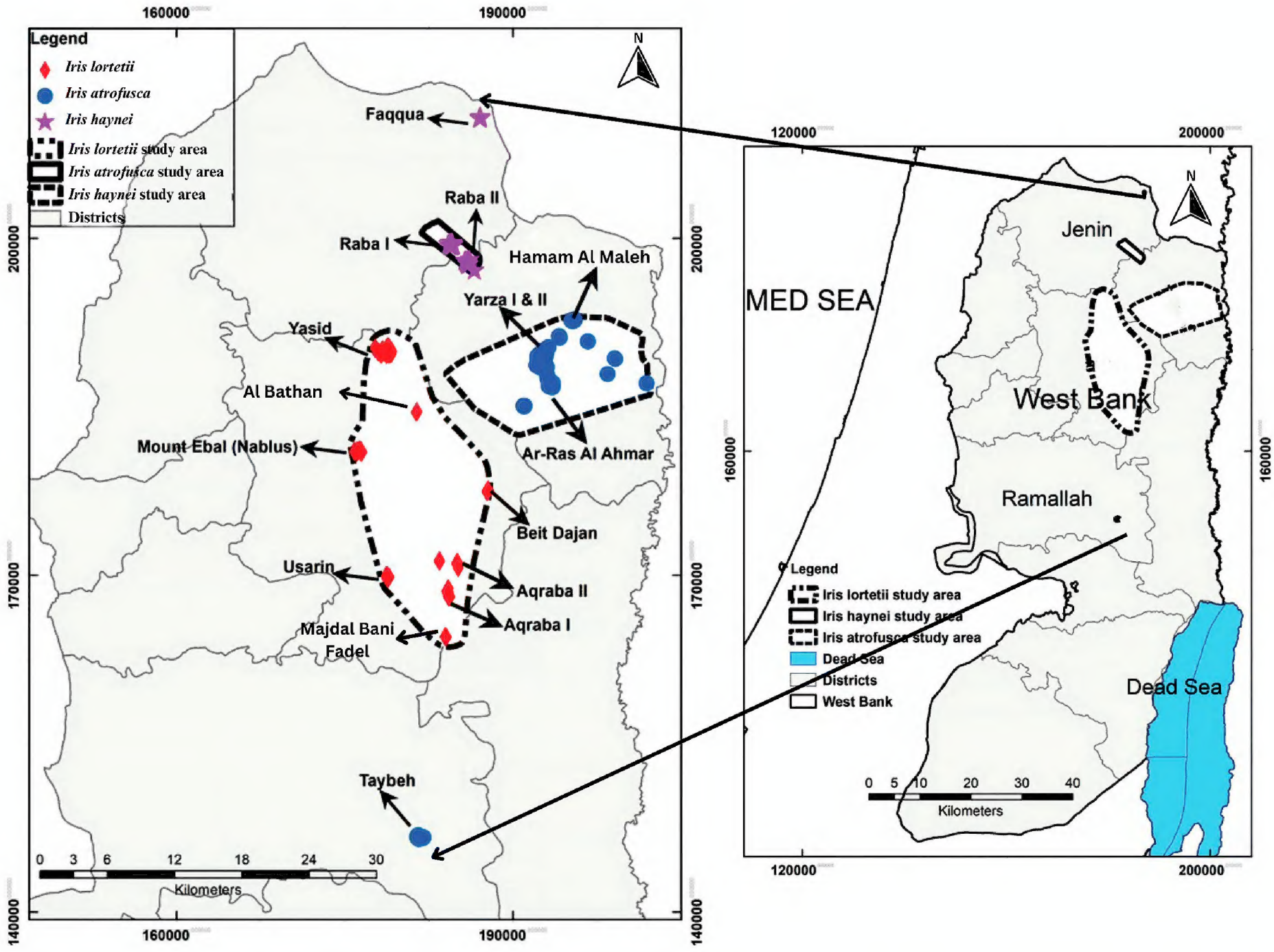

Figure 1. A. Study areas of *I. atrofusca*, *I. haynei*, and *I. lortetii* species which consisted of 355 km^2 in the northeastern region of the West Bank of Palestine. The study area of *I. atrofusca* covers the middle-central parts of Tubas district as well as the site of Taybeh village in the east-south of Ramallah district. The study area of *I. lortetti* is located in the east-central parts of the Nablus district, while the study area of *I. haynei* is located in the south-eastern part of Jenin district as well as the site of Faqqua-Jalboun area in the east-northern part of Jenin district. **B**. Distribution of *I. atrofusca*, *I. lortetii*, and *I. haynei* within the studied areas, in addition, the name and location of each locality is indicated.

GPS coordinates in the form of latitude and longitude were taken (in Decimal Degrees, DD), number of clumps, clones, and flowers of Royal Irises were counted and documented. The data underpinning the analysis reported in this paper (Inflorescence Color Variations of the three Royal Irises) are deposited at "Data repository" at https://doi.org/10.6084/m9.figshare.26485783.

When a unique color for any inflorescence was observed, the color variance is described on site. Several photographs of each color variance were taken for further examination. Anthropogenic and environmental threats causing negative impacts on the Royal Irises were recorded as well. Geographic Information System (GIS) software and Google Earth were used to determine the distribution range of recorded clones, to generate the delineation for each site, and to generate maps. In many cases, the documented clones aggregated in small patches or areas, therefore GPS coordinates for each identified clone were impossible to measure, as the best accuracy obtained in the field was about 3.4 m. Therefore, the number of GPS points represents, in most cases, the number of clumps. Consequently, the number of identified clones exceeded in several folds (in most cases) the number of recorded GPS coordinates (see results section for more details). The field survey was performed by three to twelve individuals, including team leaders. The team was divided into two to four groups, where each investigated area was divided into several small sites (depending on the total area under investigation and the number of surveyors), and each group of volunteers was assigned a specific site to ensure the coverage of the whole area, and to avoid duplication of records.

c) Royal irises description

I. lortetii Barbey (Nablus' Iris) is a sub-endemic (Feinbrun-Dothan 1986; Danin 2015), endangered species under criteria *B1ab* (*i,ii,iii,iv*)+*2ab*(*i,ii,iii,iv*) of the IUCN Red List (Sapir 2016b). It is an erect bearded rhizomatous spring-flower (Oncocyclus) with a compact rootstock. Its flowers have fall petals that are heavily impregnated with contrasting darker markings. The leaves are entire linear with smooth margins, found in fan-like clusters of 6–8 light-green leaves, more or less equaling the stems (up to 50 cm). Flowers are borne singly, its fall petals are 50–80 mm, unlined, deflexed, and displays a remarkable variety of colors ranging from pale peach to light purple, heavily suffused with very fine maroon spots, with brownish-red, sparsely red-hairy near its falls' base (Feinbrun-Dothan 1986), its standards are 90 mm–11 cm that is circularly erected, pinkish-white with dark pinkish veins; stigmas are maroon, horizontal with deflexed crests (Fig. 2A).

I. atrofusca Baker (Shafa Al-Ghor Iris), is a sub-endemic (Feinbrun-Dothan 1986; Danin 2015), vulnerable species under criteria *B1ab* (*ii,iii,iv,v*) + *2ab* (*ii,iii,iv,v*) of the IUCN Red list (Sapir 2016a). It is an erect 20–30 cm tall, bearded rhizomatous spring-flower, with stout and compact rhizomes; the leaves are entire with smooth margins, found in 5–8 fan-like clusters, and its blade-shaped leaves are 1 cm board, reaching and sometimes exceeding the base of 7–8 cm spathe. Flowers are borne singly per stem; its fall petals range size is 6–7.5 * 3–4.5 cm, recurved from about the middle, dark brown-purple to nearly black; hairs of beard are yellowish with brown tips found on the cream-colored base; its standards are erectly incurved 7–9*4.5–7 cm (Feinbrun-Dothan 1986), with dark purple-brown to dark purple radiating veins and dense dots (Fig. 2B for the morphological features of *I. atrofusca*). Flowers last for almost 5 days and produce fruit capsules, which dry and split open to release 30–50 globose seeds (Avishai and Zohary 1980).

The third investigated Royal Iris is *Iris haynei* Baker (Faqqua Iris), an endemic (Feinbrun-Dothan 1986; Danin 2015; Thorogood 2019) and vulnerable floral species under criteria *A3c*; *B1ab* (*ii,iii,v*)+*2ab* (*ii,iii,v*) of the IUCN Redlist (Sapir 2016c). It is an erect 35–50 cm tall bearded rhizomatous spring-flower with very short-creeping rhizomes that lack stolons. Its leaves are entirely with smooth margins, light-green blade-shaped found in 5–6 (8) fan-

Figure 2. A–C. Typical inflorescence coloration of *I. lortetii*, *I. atrofusca*, and *I. haynei*, respectively.

like clusters, which reach up to 40 cm tall and are typically basal (Feinbrun-Dothan 1986). Flowers are borne singly with a pair of bract-like leaves near their base; their fall petals range from 70 mm to 80 mm, which is dark purplish-lilac with a yellowish beard; their standards are 90 mm–10 cm, which ranges from dark to pale purplish-lilac color and are strongly bent from the middle (Fig. 2C).

Results

The floristic survey was conducted on a total area of 355 km^2, in the north-central parts of the West Bank, within Jenin, Tubas, Nablus, and Ramallah districts. The results and findings are summarized below:

1) Royal Iris species

1.1 *Iris lortetii*

Populations of *Iris lortetii* were documented in 1.36 km^2, distributed into ten sites, ranging from 0.981 dunams (0.000981 km^2) within Al Bathan I site to 910.8 dunams (0.9108 km^2) within Yasid site (Table 1, Fig. 1A, B). The total number of recorded *I. lortetii* clumps was 3107, representing 12625 clones and 25088 flowers, within a total surveyed area of 219 km^2, including Yasid, Usarin, Aqraba, Mount Ebal (Nablus), Majdal Bani Fadel, Al Bathan and Bayt Dajan, in Nablus district. The Yasid area (the largest studied site) was found to hold the highest number of observed *I. lortetii*, where 5714 clones and 6402 flowers were documented (Table 1, Fig. 1B, 2). Many clones were still juvenile (small; non-flowering fan-like cluster), which partially explains the low number of recorded flowers in comparison to the number of clones. On the other hand, *I. lortetii* recorded at Aqaba II site are densely aggregated within a relatively small area of only 68 dunams (0.068 km^2), having 1845 clones and 8593 flowers, indicating that the majority are adult clones (flowering cluster). In addition, *I. lortetii* was documented at an elevation of 772 m unlike the provided upper elevation limit of 600 m (IUCN 2016). Moreover, 12 unique inflorescence-coloration variances in *I. lortetii* were documented among recorded clones. The variance in coloration pattern of localities within Mount Ebal (Nablus) is the highest (17 shades of inflorescence-coloration variances) of which only 2 are of unique colorations, followed by Usarin (15) of which 5 are of unique colorations, Yasid (9) of which 3 are of unique colorations, Aqraba II (7) of which one is of unique colors, Aqraba I (2) of which one of them is of unique color, and finally Majdal Bani Fadel where only one inflorescence-coloration variance was documented. These variances range from the extremely off-white tinged with pale yellow standard petals accompanied with very pale orange fall sepals and adorned with minor spots, to the other extreme of amethyst purple standard petals, tinted with brown veins at the base with spicy brown fall sepals, which are heavily adorned with sangria dots (see Table 2 for detailed description and Fig. 4A–L) for representative images of the 12 unique inflorescence-coloration variances).

1.2 *Iris atrofusca*

Clones of *I. atrofusca* were found in 1.331 km^2, distributed among thirteen sites, including the *I. atrofusca* Ex-situ conservation site that was established in 2021, which is located at the eastern part of Mount Tammoun nature reserve. The Ex-situ site includes 106 clumps that were translocated from different sites identified as threatened ones (Table 3, Fig. 1A, B). Populations of *I. atrofusca* were found in sites ranging in size from 0.17 dunams (0.00017 km^2) (Wadi Firan) up to 493 dunams (0.493 km^2) within Khirbet Yarza site II (Table 3, Fig. 1B, 3). The total number of documented clumps was 1379, while the number of counted clones and flowers was 1853 and 8460, respectively. Khirbet Yarza (sites I to IV, 0.92 km^2) holds 1018 clones and 6239 flowers, about 55% of the total counted clones and 74% of counted flowers, of all sites (see Table 3 for more details), followed by Khirbet Ar-Ras Al Ahmar and Hamam Al Maleh that hold 342 clones with 753 flowers and 143 clones with 371 flowers, respectively. Several sites, such as Khirbet Makhul and Khirbet Samra areas, with few reported clones, were not comprehensively surveyed, because these sites are located within Israeli military zones or near Israeli settlements. Moreover, twelve unique variations in coloration of inflorescences in *I. atrofusca* were documented, ranging from the extreme white standard petals and light

Table 1. Study sites of *I. lortetii* species, coordinates, area, number of clumps, counted clones and flowers.

No	Site	Area (Dunams)- km^2	Longitude, Latitude	# of clumps	# of clones	# of flowers
1	Yasid	910.808- (0.910808 km^2)	35.30021, 32.302374	1114	5714	6402
2	Aqraba I	201.187- (0.201187 km^2)	35.35993, 32.108571	511	783	3119
3	Usarin	68.278- (0.068278 km^2)	35.30282, 32.121521	573	3012	4976
4	Aqraba II	67.958- (0.067958 km^2)	35.369195, 32.130855	543	1845	8593
5	Jabal Nablus	66.329- (0.066329 km^2)	35.27485, 32.223197	225	1080	1592
6	Beit Dajan	22.66- (0.02266 km^2)	35.397564, 32.190789	76	111	198
7	Aqraba III	12.648- (0.012648 km^2)	35.35210, 32.134567	31	37	98
8	Majdal Bani Fadel	6.514- (0.006514 km^2)	35.35767, 32.073482	28	31	76
9	Al Bathan I	3.094- (0.003094 km^2)	35.330517, 32.254045	6	11	34
10	Al Bathan II	0.981- (0.000981 km^2)	35.365886, 32.230081		2	5
Total		**1360- (1.36 km^2)**		**3107**	**12624**	**25088**

Table 2. *Iris lortetii* variations in coloration of inflorescence, with a description of color variation and its spatial location.

No	Description of variation in coloration of inflorescences	Latitude, Longitude
1	Brown fall sepals which are adorned with numerous maroon spots and pale lilac standard petals **	32.076481, 35.356396
2 (A)	Lilac standard petals with sepia veins; brown fall sepals that are heavily adorned with burnt umber spots, as well as tinged red inner canals*	32.105827, 35.36034
3	Very light heliotrope standard petals with cinnamon brown fall sepals which are heavily adorned with sangria dots**	32.109192, 35.360042
4 (B)	Pale rosy standard petals with light brownish background which is heavily adorned by reddish brown dots*	32.120067, 35.302369
5	Light lilac standard petals with very light brown fall sepals which are heavily adorned with brown dots	32.120094, 35.302956
6	Yellow standard petals with pastel orange fall sepals which are moderately adorned with maroon dots	32.120117, 35.302333
7 (C)	Light lilac standard petals with medium brown fall sepals and very dark background beneath the beard, mildly adorned by maroon dots*	32.120167, 35.302372
8	Light lilac standard petals with very light brown yellow fall sepals which are heavily adorned with brown dots	32.120182, 35.302349
9	Light lilac standard petals with very light brown fall sepals which are heavily adorned with very dark violet dots**	32.1203, 35.302238
10	Within the same clone there were flowers of light lilac standard petals and light purple fall sepals as well as light lilac standard petals and pink tinged with yellow fall sepals flower	32.120317, 35.303283
11	It is a typical Iris lortetii flower color, but its clone is relatively very tall reaching 65 cm	32.120467, 35.303133
12	Very light lilac standard petals that are tinted by brown at its base with orangish yellow fall sepals which are scarcely adorned with dots**	32.1215, 35.303183
13 (D)	An off-white light pastel yellow standard petal, with very pale orange fall sepals adorned with minor spots*	32.121511, 35.303167
14	Lilac standard petals that are heavily veined with yellow veins, as well as to yellowish brown fall sepals that are adorned by brown spots	32.121685, 35.303501
15	Light lilac standard petals with light brown fall sepals that are heavily adorned with brown spots**	32.121767, 35.303497
16 (E)	Light lilac standard petals with light lilac fall sepals, which are moderately adorned with dark purple dots upon a dark purple background*	32.121769, 35.3035
17	Light lilac standard petals with very light-yellow tinted with brown fall sepals which are heavily adorned with brown dots	32.122292, 35.30164
18	Light lilac standard petals with light purple fall sepals that are adorned with maroon spots*	32.122567, 35.30255
19	Very light pink standard petals with very light yellow tinted with orange fall sepals which are mildly adorned with minute maroon dots**	32.13089, 35.369011
20	Very light lilac standard petals with light lilac fall sepals that are adorned with minor purplish dots	32.131778, 35.369353
21 (F)	Light lilac standard petals with light purple fall sepals which are heavily adorned with maroon spots*	32.131789, 35.368597
22	Very light purple standard petals with cinnamon brown fall sepals, which are moderately adorned with royal maroon dots, having a very dark burgundy background beneath the beards and tunnel entrance	32.131797, 35.369417
23	Very light lilac standard petals with medium brownish fall sepals that are heavily adorned with purple dots**	32.131805, 35.369427
24	Very light lilac standard petals with medium brownish fall sepals that are adorned with minor purple spots	32.131903, 35.3689
25	Light heliotrope purple standard petals with brown fall sepals which are moderately adorned with sangria marron dots**	32.132014, 35.369078
26	Light pinkish lilac standard petals with brown fall sepals which are heavily adorned with maroon dots**	32.216022, 35.271748
27 (G)	Light lilac standard petals with lilac fall sepals that are tinted with brown and heavily adorned with dark amethyst dots	32.222055, 35.278806
28	Medium pastel lilac (Pantone) standard petals with light brownish fall sepals which are heavily adorned with dark maroon spots	32.222355, 35.271603
29	Light periwinkle lilac standard petals which are heavily veined with light brown fall sepals which are lightly adorned with chestnut brown dots**	32.222358, 35.271539
30	Light lilac standard petals with dark purple veins, which are mildly tinted brown at their base with very light caramel brown fall sepals that are heavily adorned with light brown coffee brown spots**	32.222361, 35.271603
31	Light lilac standard petals and light pastel brown fall sepals which are heavily adorned with dark amethyst dots**	32.222436, 35.2785
32 (H)	Light lavender lilac standard petals with very light pale chestnut fall sepals which are adorned with sangria dots*	32.222447, 35.271408
33	Very light lilac standard petals that are tinted with medium brown veins; very light sandy brown fall sepals which are moderately adorned with very dark brown spots**	32.222518, 35.271957
34	Very light lilac standard petals, which are tinted brown at their base with light pale chestnut fall sepals heavily adorned with sangria dots**	32.222519, 35.271358
35 (I)	Very light lilac standard petals with very light cinnamon brown fall sepals which are mildly adorned with chestnut brown spots*	32.2226, 35.272089
36	Light lilac standard petals that are tinted with brown veins with light brown fall sepals, which are heavily adorned with dark purplish dots**	32.22325, 35.277028
37	Light lilac standard petals which are moderately tinted by pale yellowish brown at their base and light brown fall sepals which are heavily adorned with sangria dots	32.223251, 35.277027
38	Lilac standard petals with heliotrope purple fall sepals which are adorned with sangria dots**	32.223343, 35.276718
39	Light periwinkle lilac standard petals which are heavily veined with medium brownish fall sepals that are heavily adorned with chestnut brown dots**	32.223381, 35.277494

No	Description of variation in coloration of inflorescences	Latitude, Longitude
40	Pale lavender standard petals with very light brown fall sepals which are mildly adorned with dots**	32.223386, 35.276483
41	Light lilac standard petals with purple fall sepals which are adorned with dark amethyst dots**	32.223411, 35.276542
42	Light lilac standard petals with light pink fall sepals which are adorned with sangria dots**	32.223528, 35.276853
43	Light purple standard petals with orange veins and light brown fall sepals**	32.223568, 35.276337
44	Light lilac standard petals with heliotrope purple fall sepals which are adorned with dark purple dots**	32.291477, 35.341309
45	Lilac amethyst standard petals that have notable purple veins with brown background fall sepals which are heavily dotted with burnt umber dots**	32.300201, 35.302105
46 (J)	Very light lilac amethyst standard petals with purple veins at the end and brown at the base of their venation and light brown background fall sepals which is moderately dotted with cinnamon brown dots*	32.300209, 35.302132
47 (K)	Light lilac standard petals that have orange veins with pale orange fall sepals with dark orange background beneath the beard*	32.300259, 35.302124
48	Lilac amethyst standard petals with purple veins and brown background fall sepals which are heavily dotted with umber dots**	32.30027, 35.302204
49	Very light lilac mauve standard petals with light cinnamon brown fall sepals tinted with orange beneath the beard and lightly adorned with brownish dots**	32.300301, 35.302101
50	Light lilac standard petals with medium amethyst lilac fall sepals which are heavily adorned with sangria dots**	32.300725, 35.296961
51 (L)	Amethyst purple standard petals tinted with brown veins at the base with spicy brown fall sepals, which are heavily adorned with sangria dots*	32.305214, 35.291336

Note: * 12 unique *Iris lortetii* variations in coloration, ** 25 different hues of these 12 unique variations with minor differences, the remaining 14 flowers are simply similar shade of color as their subsequent peers, but documented in different sites.

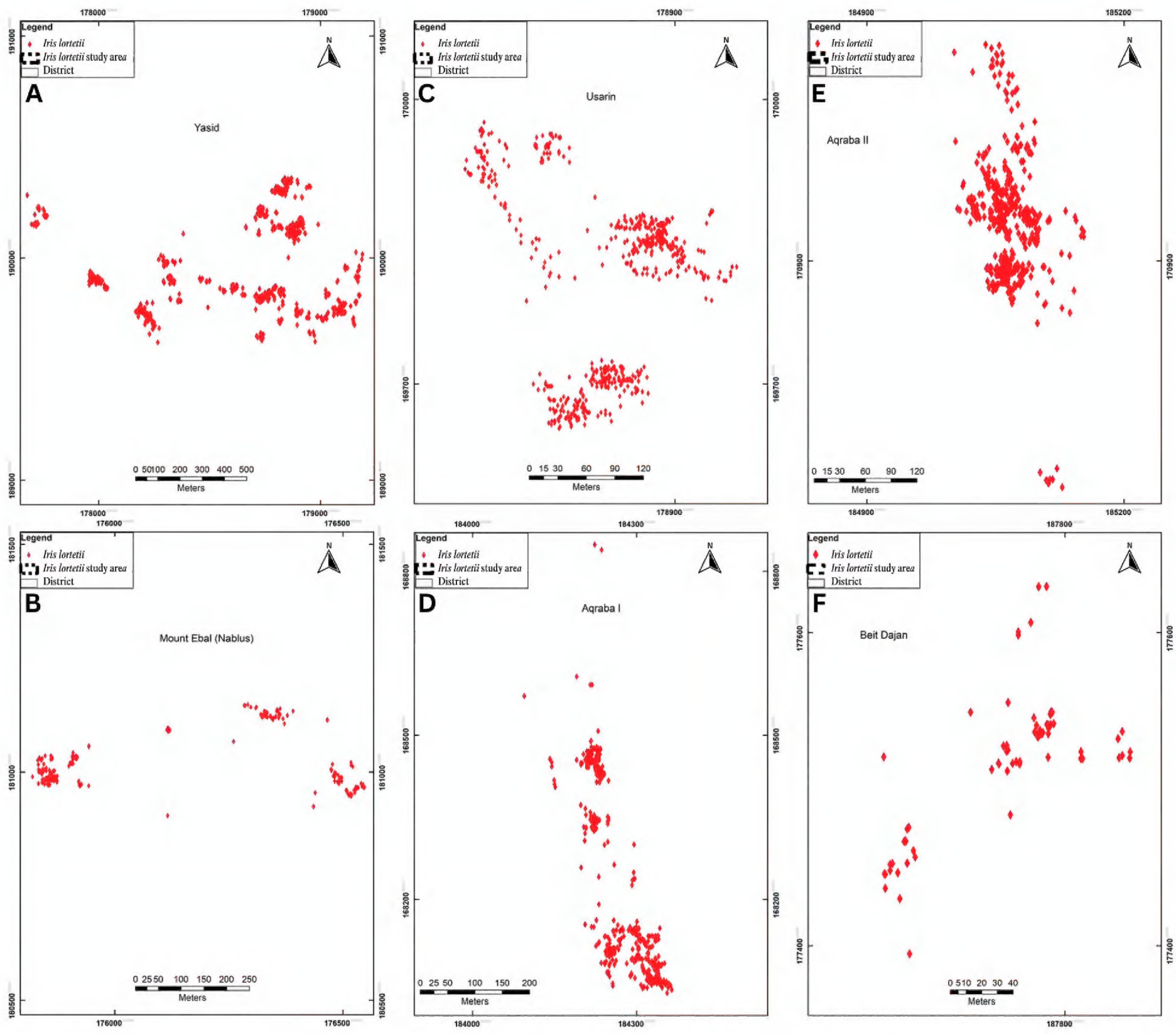

Figure 3. Distribution pattern and densities of *I. lortetii* in six explored sites (from **A** to **F**) of high population densities, with the study area. **A.** Yasid; **B.** Mount Ebal (Nablus); **C.** Usarin; **D.** Aqraba I; **E.** Aqraba II; **F.** Beit Dajan.

Figure 4. Different variations in coloration of *I. lortetii* inflorescences. Images from **A** to **L** depict the inflorescence colorations of *I. lortetii* numbers 2A, 4B, 7C, 13D, 16E, 21F, 27G, 32H, 35I, 46J, 47K, and 51L in Table 2, respectively.

plumpish purple fall sepals that are heavily veined with plum purple veins, to pale yellow-orange fall sepals, with maximum purple standard petals which are tinted, orange at their base with dark brown background beneath an orangish beard (see Table 4 for detailed description and Fig. 6A to I for representative images of nine unique inflorescence-coloration variances).

1.3 *Iris haynei*

Populations of *I. haynei* were discovered across a total surveyed area of 9.548 km^2, distributed among four sites, ranging in size from 1.7 dunams (0.0012 km^2) (Raba III site) to 254.2 dunams (0.2542 km^2) (Raba I site, refer to Table 5 and Fig. 7 for more details). In the sites of Raba and Faqqua, a total of 1815 clumps were recorded, representing 10729 *I. haynei* clones and 9562 flowers. However, it's anticipated that the actual number of *I. haynei* clones could be higher, especially considering that the Faqqua-Jalbun area was not comprehensively surveyed, except to a specific site (see discussion for more details). Raba areas (sites I, II, and III), held high population densities of *I. haynei*, which have never been recorded before. Furthermore, 7 unique and rare variations in coloration of inflorescences were detected. These distinct inflorescence colorations range from light mauve standard petals with sandy brown fall sepals, finely veined with woody walnut brown veins, to very pale lilac standard petals with pale rosy background falls, moderately adorned with numerous mildly lilac dots, highlighting a notable feature

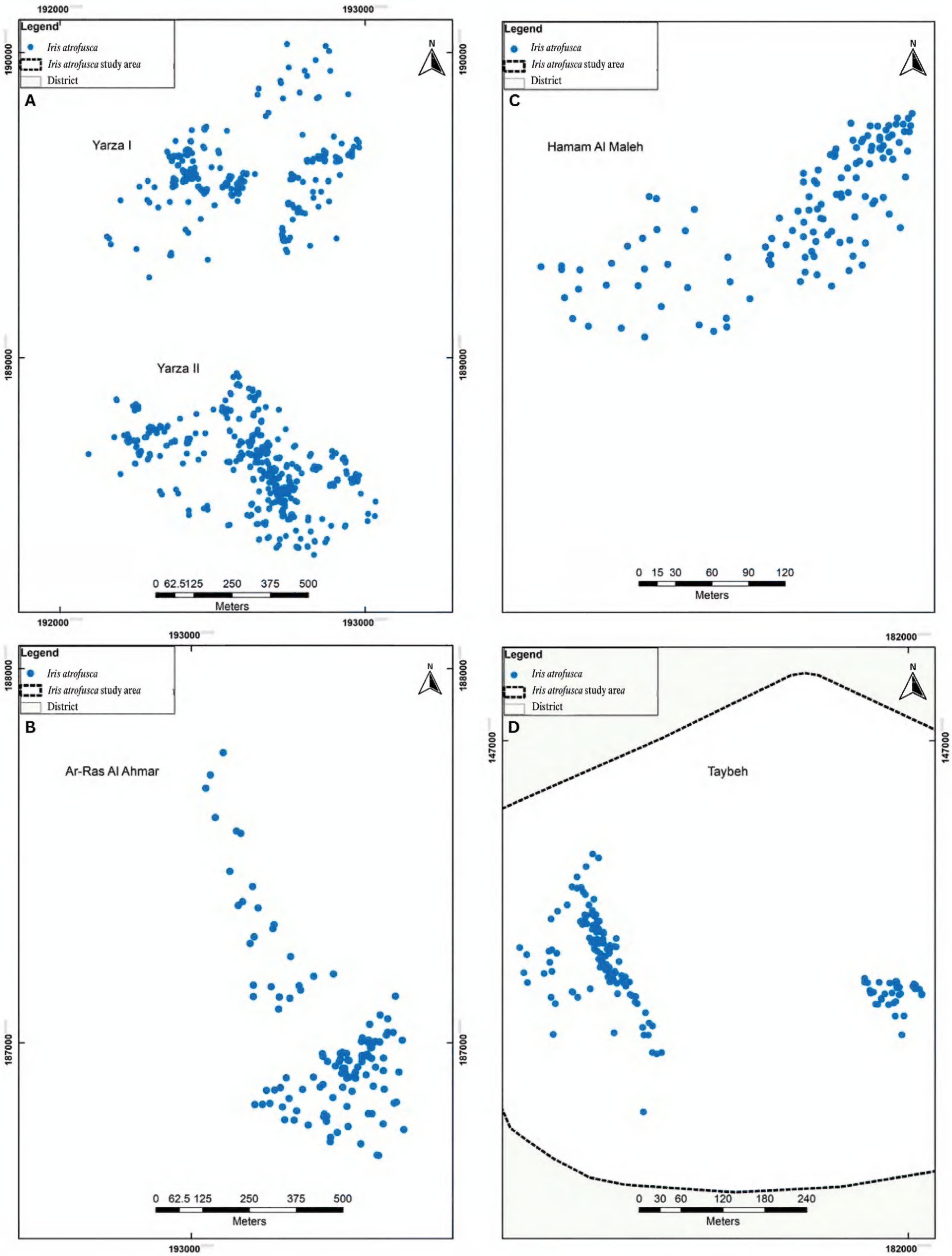

Figure 5. Distribution pattern and density of *I. atrofusca* in 4 explored sites (from **A** to **D**) of high population densities, with the study area. **A.** Yarza I and Yarza II; **B.** Ar-Ras Al Ahmar; **C.** Hamam Al Maleh; **D.** Taybeh. For more details see Table 3.

of this species. Other recorded inflorescence-color variations include white standard petals which are tinted with a pale amber hue accompanied with light amber orange fall sepals (refer to Table 6 for detailed description and Fig. 8A–E for representative images of 5 unique inflorescence-coloration variances).

Figure 6. Variations in coloration of *I. atrofusca* inflorescences. Images from **A** to **I** depict the inflorescence coloration of *I. atrofusca* numbers 2A, 5B, 6C, 8D, 9E, 10F, 12G, 13H, and 14I in Table 4.

Table 3. Study areas of *I. atrofusca* species; central GPS coordinates (in Decimal Degrees), total area, number of clumps, counted clones, and flowers.

No	Site Name	Area (Dunams)- km²	Latitude, Longitude	# of clumps	# of clones	# of flowers
1	Khirbet Yarza I	414.546- (0.414546 km²)	32.29108, 35.448822	833	1018	6293
2	Khirbet Yarza II	492.641- (0.492641 km²)	32.29869, 35.448809			
3	Khirbet Yarza III	11.618- (0.011618 km²)	32.313986, 35.465814			
4	Khirbet Yarza IV	0.887- (0.000887 km²)	32.305928, 35.455605			
5	Khirbet Ar-Ras al Ahmar	279.371- (0.279371 km²)	32.276278, 35.456899	126	342	735
6	Hamam al Malih	44.831- (0.044831 km²)	32.32738, 35.478742	126	143	371
7	Khirbet Samra	0.931- (0.000931 km²)	32.310811, 35.492787	3	17	20
8	Taybeh I	59.482- (0.059482 km²)	31.912178, 35.331596	176	218	697
9	Taybeh II	6.975- (0.006975 km²)	31.912227, 35.336104			
10	Wadi Firan	0.171- (0.000171 km²)	32.276788, 35.549023	2	2	10
11	Khirbet Makhul I	0.225- (0.000225 km²)	32.284242, 35.511314	3	3	11
12	Khirbet Makhul II	2.005- (0.002005 km²)	32.296504, 35.51824	4	4	28
13	Jabal Tammoun	14.545- (0.014545 km²)	32.258630, 35.432075	106	106	295
	Total	**1328.228- (1.328228 km²)**		**1379**	**1853**	**8460**

Table 4. *Iris atrofusca* variations in coloration of inflorescence, with a description of color variation and its location.

No	Description of variation in coloration of inflorescences	Latitude, Longitude
1	Dark brown-purple to nearly black fall sepals, ahead with yellowish hairs of beard. Its standard petals are incurved with dark purple radiating veins and dense dots. The species was found in Wadi Firan at elevation of 209 *mbsl*	32.277061, 35.547375
2 (A)	White standard petals with light plumpish purple fall sepals that are heavily veined with plum purple veins	32.29064, 35.45058
3	Three different colors are detected within the same cluster of clones which display a white standard petal with light pastel orange fall sepal's flower upon a white background, whereas on the same clone, it has another inflorescence with dark brown to nearly black fall sepals; while its standard petals are erectly incurved with dark purplish-brown radiating veins and dense dots. In addition, adjacent to them there is yet another color variation clone with dark purplish fall sepals and its standard petals are dark purple with radiating veins and dense dots.	32.290709, 35.450457
4	White standard petals with light pastel magenta fall sepals upon a white background, whereas in the same clone, there is dark brown-purple to nearly black fall sepals; its standard petals erectly incurved with dark purplish-brown to dark purple radiating veins and dense dots.	32.290728, 35.450367
5 (B)	Pale yellow-orange fall sepals, with maximum purple standard petals which are tinted orange at their base with a dark brown background beneath an orangish beard	32.299589, 35.447436
6 (C)	Light lilac standard petals accompanied by light sepia fall sepals with a black background beneath the beard	32.299517, 35.451044
7	Cerise standard petals accompanied by dark pink fall sepals with black background beneath the beard	32.292731, 35.44871
8 (D)	Off- White standard petals which are heavily veined with caramel veins with brunette fall sepals	32.258559, 35.432473
9 (E)	Boysenberry standards petals which are heavily veined with black fall sepals	32.300137, 35.451813
10 (F)	Off- White standard petals which are heavily veined by russet veins and tinted apricot shade at the base with burnt orange fall sepals that are heavily veined by clay veins	32.299517, 35.451044
11	Light lavender standard petals tinted with purple-brown hue at the base and sepia fall sepals	32.299069, 35.449207
12 (G)	Wine Dregs colored standard petals with bordeaux colored fall sepals	32.328068, 35.47974
13 (H)	Brandy colored standard petals that are tinted burnt umber at the base and heavily veined by chestnut veins with russet fall sepals	32.291222, 35.449256
14 (I)	Purple wine standard petals with tyrian purple fall sepals	32.327432, 35.479189

Table 5. Studied areas of *I. haynei* with their central GPS coordinates in the form of latitude and longitude were taken in the form of Decimal Degrees (DD), total area, number of clumps, counted clones, and flowers.

No	Site	Area (Dunams)- km²	Longitude, Latitude	# of clumps	# of clones	# of flowers
1	Raba I	254.212- (0.254212 km²)	35.363602, 32.389039	11	59	126
2	Raba II	107.256- (0.107256 km²)	35.378278, 32.375270	790	3281	5057
3	Raba III	1.726- (0.001726 km²)	35.385375, 32.368365	971	7276	4201
4	Faqqua	21.551- (0.021551 km²)	35.391059, 32.490827	43	113	178
Total		**385- (0.385 km²)**		**1815**	**10729**	**9562**

Table 6. *I. haynei* variation in coloration of inflorescences, with a description of color variation and its location.

No	Description of variation in coloration of inflorescences	Latitude, Longitude
1 (A)	Light mauve standard petals with sandy brown fall sepals which are finely veined with walnut brown veins	32.388721, 35.365295
2 (B)	Light periwinkle standard petals which are heavily veined with dark purple heather veins and have light sandy brownish fall sepals which are finely veined with very light peanut brown veins	32.388786, 35.365181
3 (C)	A very pale lilac standard petals with pale rosy background fall sepals which are moderately adorned with numerous mildly lilac dots	32.388901, 35.365231
4 (D)	An off-white standard petal with very light brown fall sepals with a brownish background beneath the beard	32.485872, 35.401144
5 (E)	White standard petals which are tinted with pale amber hue accompanied with light amber orange fall sepals	32.48735, 35.39579
6	Light lilac standard petals accompanied with medium lilac fall sepals	32.48214, 35.40364
7	White standard petals which are tinted with pale lemon-yellow hue accompanied with lemon yellow fall sepals	32.48587, 35.40114

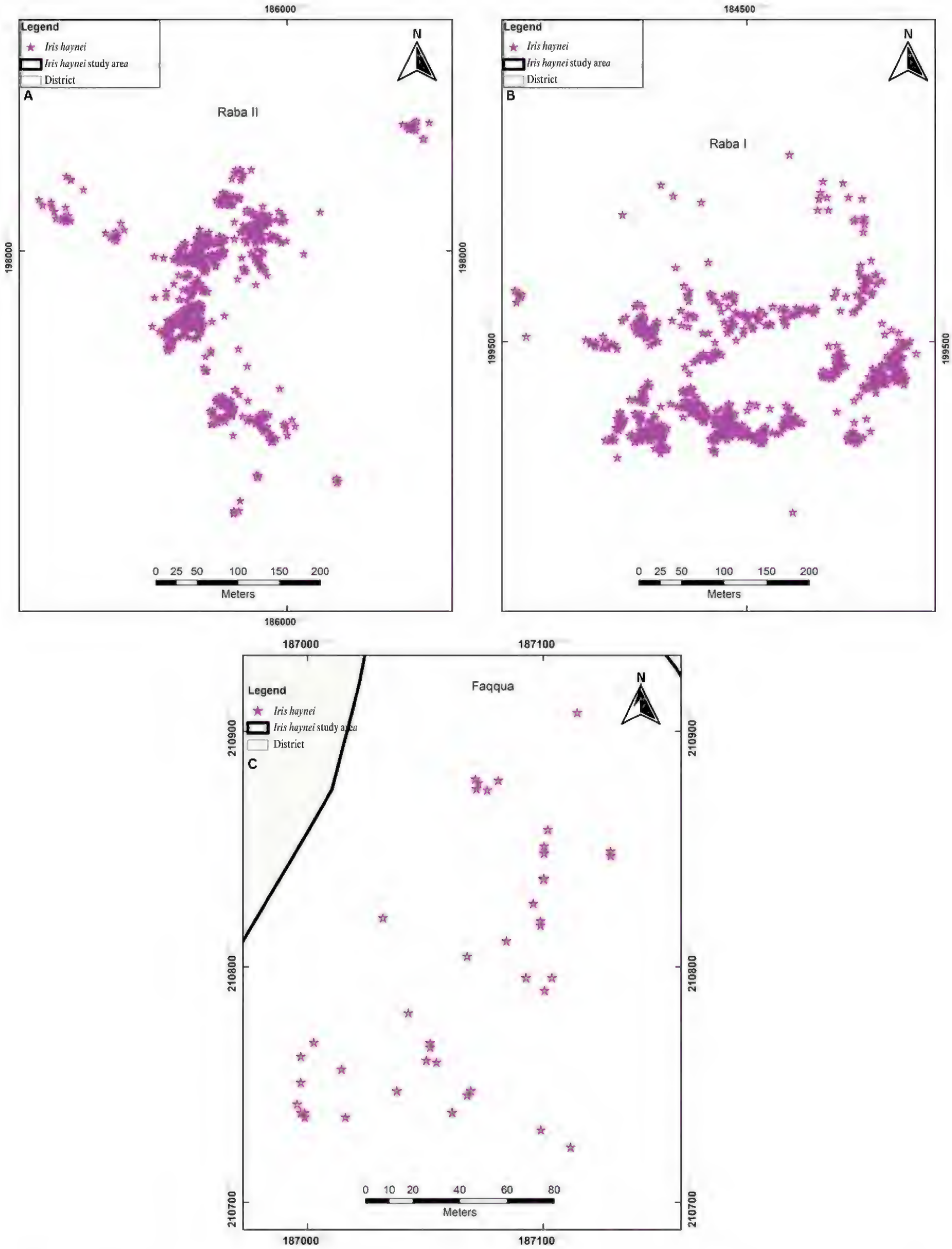

Figure 7. Distribution pattern and density of *I. haynei* in 3 explored sites (from **A** to **C**) of high population densities, within the study area. **A.** Raba I; **B.** Raba II; **C.** Faqqua. For more details see Table 5.

2) Habitat description of studied Royal Irises species

2.1 *Iris lortetii*

All nine studied sites of *I. lortetii's* habitats share two mutual ecosystem units, the montane Mediterranean vegetation (woodlands, garrigue), and transition shrublands along the east-northern parts of the central mountains within Nablus governorate.

2.1.1 Yasid area:

Yasid is located 15 km northeast of Nablus city. The western-central area of Yasid is considered a Mediterranean maquis. On the other hand, its east-central side is more semi-

Figure 8. Variations in the coloration of *I. haynei* inflorescences. Images from **A** to **E** depict the flower coloration of *I. lortetii* number 1A, 2B, 3C, 4D, and 5E in Table 6.

dry rocky garrigue with scattered shrubs and chamaephytes adapted to the Mediterranean climate, where most of the *I. lortetii* clones were observed (see Fig. 9A). The area used to be a traditional agricultural land where several cereal crops were cultivated a few decades ago. The main vegetation type of the studied area consists of *Crataegus aronia* (L.) DC., *Styrax officinalis* L., *Amygdalus communis* L., *Sarcopoterium spinosum* (L.) Spach, *Gundelia tournefortii* L., *Lomelosia palaestina* (L.) Rafin, the rare *Asphodeline lutea* (L.) Rchb., *Phlomis viscosa* Poir., the sub-endemic *Centaurea cyanoides* Berggren & Wahlenb., medicinal sage (*Salvia officinalis* L.) and the sub-endemic *Salvia hiérosolymitana* Boiss. The terrain and elevation of the area create microclimates that support different types of Mediterranean floral species such as rocky cliff slopes that are the habitat of the sub-endemic *Onopordum cynarocephalum* Boiss. & Blanche.

2.1.2 Mount Nablus I (Mount Ebal):

Mount Nablus is considered one of the highest mountains (935 *mamsl*) in the West Bank and the highest in Nablus district. The site is characterized by very steep, rocky cliffs, and garrigue habitats of limestone soil with scattered shrubs and chamaephytes, which are adapted to the Mediterranean climate (see Fig. 9F). These plant species include *Crataegus aronia*, the rare *Asphodeline lutea*, *Scrophularia xanthoglossa* Boiss, *Scolymus hispanicus* L., and the sub-endemic *Onopordum cynarocephalum* Boiss. & Blanch. In addition, the upper southern part vegetation of the mountain includes scattered pine trees surrounding the *I. lortetii* population, which is a remnant of a man-made coniferous forest.

2.1.3 Beit Dajan

Beit Dajan is located in the eastern part of Nablus district. The site's habitats are characterized by its semi-dry, garrigue rocky hills of limestone and dolomite soil, to semi-steppe shrubland with scattered shrubs and chamaephytes of *Crataegus aronia*, *Acanthus syriacus* Boiss., *Sarcopoterium spinosum*, *Gladiolus italicus* Mill. and the rare *Asphodeline lutea*, in addition to the vulnerable and rare *Petrorhagia zoharyana* A.Liston.

Figure 9. Images for six of the nine described habitats of *I. lortetii*; **A.** Yasid area – Nablus district; **B.** Majdal Bani Fadel – Nablus district; **C.** Aqraba I (Khirbet Al Tawil) – Nablus district; **D.** Aqraba II – Nablus district; **E.** Usarin – Nablus district; **F.** Mount Nablus (Mount Ebal) – Nablus district.

2.1.4 Aqraba (Aqraba I, Aqraba II, & Aqraba III)

Aqraba is located in the southeastern parts of Nablus district, where three sites of *I. lortetii* were located:

• Aqraba I (Khirbet Al Tawil)

The site is located on the east south of Aqraba village. It is characterized by its semi-steppe shrubland habitat, which falls upon shrub-steppe rocky montane of limestone chalk and marl dominated by scattered shrubs (see Fig. 9C). The main vegetation of the site consists of *Calicotome villosa* (Poir.) Link, *Sarcopoterium spinosum*, *Rhamnus lycioides* L., *Fumana arabica* (L.) Spach, *Fumana thymifolia* (L.) Webb, as well as the rare geophyte *Anacamptis collina* (Banks & Sol. ex Russell) R.M.Bateman, Pridgeon & M.W.Chase. This site is penetrated by the Irano-Turanian chorotype due to the occurrence of various populations of the *Matthiola longipetala* (Vent.) DC. Moreover, the documentation of the endemic *Matthiola aspera* Boiss. and *An-*

chusa aegyptiaca (L.) DC. within the site indicates that the site is also penetrated by the Saharo-Arabian chorotype.

• Aqraba II

The habitat of Aqraba II site is characterized by its rocky-stony montane garrigue of limestone chalk and marl soil mixed with the Mediterranean batha habitat (see Fig. 9D). It is penetrated by both the Irano-Turaninan and the Saharo-Arabian chorotypes due to the presence of the sub-endemic *Trichodesma boissieri* Post and the endemic *Matthiola aspera*, which both grow in large populations mainly in the slopes of the Nablus Desert. Its main vegetation is dominated by *Sarcopoterium spinosum*, *Alcea setosa* (Boiss.) Alef., the sub-endemic *Salvia hierosolymitana*, *Alkanna strigosa* Boiss. & Hohen., *Convolvulus dorycnium* L., the very rare *Alcea digitata* (Boiss.) Alef., and the *Salvia dominica* L. which spreads from the Mediterranean chorotype to the Irano-Turanian. It is worth mentioning that this site is dominated by an extremely dense population of the sub-endemic *I. lortetii* with the highest record of *I. lortetii*'s flowers among the other studied sites, likely due to minor anthropogenic effects.

• Aqraba III

This site is found in the northeastern part of Aqraba town. It is characterized by its semi-steppe garrigue olive orchard habitat with the main vegetation of *Olea europaea* L., *Silybum marianum* (L.) Gaertn., and *Drimia aphylla* (Forssk.) J.C. Manning & Goldblatt. The site endured several anthropogenic effects including urbanization, habitat destruction, and livestock overgrazing, which is reflected in a decreased and scattered number of clones.

2.1.5 Usarin:

Usarin is located to the southeast of Nablus district. It is characterized by its semi-steppe shrubland and rocky montane phrygana of limestone chalk and marl soil (see Fig. 9E). Its main vegetation includes *Pistacia terebinthus* L., *Olea europea*, *Calicotome villosa*, *Sarcopoterium spinosum*, *Jasminum fruticans* L., *Linum mucronatum* Bertol., *Linum corymbulosum* Rchb., the sub-endemics *Salvia judaica* Boiss, *Salvia hierosolymitana* and *Alcea galilaea* Zohary and the *Alcea digitata* (Boiss.) Alef., of which both are classified as a very rare plant species.

2.1.6 Majdal Bani Fadel:

Majdal Bani Fadel is located to the southeast of Aqraba town, to the north of Duma village. Its habitat is characterized by transition semi-steppe shrubland with olive orchards, and brown rendzinas limestone chalk and marl soil (see Fig. 9B). The main vegetation of the site includes *Calicotome villosa*, *Sarcopoterium spinosum*, *Cistus criticus* L., *Cistus salviifolius* L. and the rare *Anacamptis sancta* (L.) R.M.Bateman, Pridgeon & M.W.Chase species. This site is an agricultural land, therefore had a minor population of *I. lortetii* clones.

2.2 *Iris atrofusca*

All studied sites, where *I. atrofusca* clones were documented, are classified as transitional shrublands along the eastern slopes of Tubas and Ramallah districts, characterized by their low vegetation coverages with scattered trees. The area suffers from several anthropogenic threats that affect *I. atrofusca's* populations, including livestock overgrazing, wild plants foraging, flower collection and uprooting, and agricultural practices.

2.2.1 Khirbet Yarza (sites I, II, III, IV) and Khirbet Al-Ras Al Ahmar

Khirbet Yarza is located 6 km to the east of Tubas city, within the east-central parts of Tubas district. It is a transition shrubland (see Fig. 10C, D) with semi-steppe, rocky slopes of clay silt, gravel (Alluvium soil), and dolomite soil with scattered shrubs of *Ceratonia siliqua* L., *Crataegus aronia*, *Rhamnus lycioides*, *Ziziphus spina-christi* (L.) Desf., and *Ziziphus lotus* (L.) Lam. The main vegetation of the sites includes *Ranunculus asiaticus* L., *Carlina curtum* Heldr. ex Halacsy, the sub-endemic *Cynara syriaca* Boiss, *Arum discordies* Sm., *Scolymus maculatus* L., *Ononis natrix* L., and the sub-endemic *Chaetosciadium trichospermum* (L.) Boiss.

2.2.2 Hamam al Malih

This hilly area is located about 4 km to the east north of Khirbet Yarza. It is a typical transition zone of rocky slopes with brown igneous and metamorphic soil (see Fig. 10A). The main reported vegetation of the area include *Crataegus aronia*, *Gundelia tournefortii*, *Acanthus syriacus*, *Anthemis pseudocotula* Boiss., *Ranunculus asiaticus* and the sub-endemic *Chaetosciadium trichospermum*, *Carthamus glaucus* M.Bieb., *Phlomis brachyodon* (Boiss.) Zohary, *Phlomoides laciniata* (L.) Kamelin & Makhm., *Androcymbium palaestinum* Baker, *Ranunculus millefolius*, *Notobasis syriaca* (L.) Cass., *Pistacia lentiscus* L., *Blepharis attenuata* Napper, *Salvia viridis* L., *Atractylis cancellata* L., *Salvia dominica* L., *Anemone coronaria* L., the endemic *Onopordum palaestinum* Eig, *Rumex cyprius* Murb., *Helianthemum salicifolium* (L.) Mill., *Ziziphus lotus*, and *Sarcopoterium spinosum*. The site's habitats are under threat of overgrazing and herding of goats, sheep, and cows, which devastate the habitats and clones of the *I. atrofusca.*

2.2.3 Wadi Firan

Wadi Firan is a relatively medium Wadi (~4.5 km) that extends from Khirbet Makhul to the Jordan River. It is a semi-steppe slope of a rocky and semi-arid canyon with brown lithosols arid chalk and marl soil (see Fig. 10B). The main

observed vegetation of the Wadi includes *Retama raetam* (Forssk.) Webb, *Noaea mucronata* (Forssk.) Asch. & Schweinf., *Tulipa agenensis* DC, the rare species of *Allium aschersonianum* Barbey, Salvia dominica, the sub-endemic *Trichodesma boissieri*, *Allium erdelii* Zucc., *Phlomis brachyodon*, *Ornithogalum narbonense* L., *Ranunculus asiaticus*, *Notobasis syriaca*, *Adonis dentata* Delile, *Scorzonera papposa* DC., *Papaver umbonatum* Boiss., *Scrophularia xanthoglossa*, *Pterocephalus brevis* Coult., *Pallenis spinosa* (L.) Cass., *Lomelosia palaestina* (L.) Rafin, and *Ziziphus lotus*.

2.2.4 Khirbet Samra

The site is a transition shrubland area, located to the northeast south of Khirbet Yarza, in the east-central parts of Tubas district. The habitat of the site is a rocky, semi-steppe hill with brown rendzinas, limestone chalk, and marl soil. The main vegetation of the area consists of *Ferula communis* L., *Carlina curtum*, *Salvia viridis*, *Salvia dominica*, *Phlomis brachyodon*, *Kickxia aegyptiaca* (L.) Nabelek, *Ziziphus spina-christi*, *Gundelia tournefortii*, *Teucrium capitatum* L., *Gypsophila capillaris* (Forssk.) C.Chr., *Sarcopoterium spinosum*, *Anemone coronaria*, *Calicotome villosa*, *Heliotropium rotundifolium* Lehm., *Moraea sisyrinchium* (L.) Ker-Gawler, *Retama raetam*, and *Ziziphus lotus*.

2.2.5 Mu'arrjat Al Taybeh

The site is in the eastern part of Ramun town, within the east-central part of Ramallah district. The study area is a dry rocky, semi-steppe slope with limestone chalk and marl soil. The main vegetation reported in the area include *Phlomoides laciniata*, *Phlomis brachyodon*, *Ridolfia segetum* (L.) Moris, the rare species of *Serratula pusilla* (Labill.) Dittrich, *Salvia palaestina* Benth., *Salvia samuelssonii* Rech.f., *Astragalus macrocarpus* DC., *Gladiolus atroviolaceus* Boiss. and *Achillea arabica* Kotschy, with the vulnerable and the rare *Petrorhagia zoharyana*.

2.3 Iris haynei

I. haynei was found in four sites, within the northeastern parts of the West Bank. All sites share a common ecosystem feature of montane Mediterranean vegetation (woodlands and phrygana), including plantations and olive groves.

2.3.1 Raba village:

It is located 11 km to the east-south of Jenin city, and 12 km to the southwest of Faqqua-Jalbun villages, in the eastern part of Jenin district. The site was divided into three main areas, namely: Raba I, II, and III, based on habitat and vegetation cover.

• Raba I site

Raba I site is in the west-southern parts of Raba town. The site is dominated by *Pinus halepensis* Mill. trees, a man-made small forest (see Fig. 11A). It is characterized by its garrigues habitat of rocky slopes and limestone soil. The main vegetation of the site includes *Pistacia terebinthus* L., *Rhamnus lycioides*, *Ruta chalepensis* L., *Ferula communis*. It is home to many geophytes such as *Ophrys umbilicate* Desf., the rare *Ophrys bornmuelleri* M.Schulze and the *Anacamptis pyramidalis* (L.) L.C.M. Richard. The forest has a widespread occurrence of *Drimia aphylla* and *Asphodelus ramosus* L., which are indicators of livestock overgrazing. In addition, the northwest part of the site is an urbanized area with agricultural fields of different orchards and cereal crops. The vegetation of this area is dominated by *Olea europaea*, *Silybum marianum*, and *Sinapis alba* L.

• Raba II & III sites

These two limestone rocky sites are located within two hilly areas, to the east south of Raba town, and to the east of Raba I site. They are characterized by their rocky shrub-steppes garrigue habitats which are intermixed with Mediterranean woodland and shrubs (see Fig. 11C). The vegetation of the two sites is dominated by *Pistacia terebinthus*, *Ruta chalepensis*, *Ceratonia siliqua*, *Jasminum fruticans*, *Sarcopoterium spinosum*, *Rhamnus lycioides*, *Ranunculus asiaticus*, the sub-endemic *Onopordum cynarocephalum*, *Ballota undulata* (Sieber ex Fresen.) Benth., and *Kickxia aegyptiaca*.

2.3.2 Faqqua site:

Faqqua is in the northeastern part of Jenin district. The investigated area within Faqqua is located in the west-southern part of Faqqua town. The site is a cultivated land with rocky garrigue (see Fig. 11B) of limestone soil mixed with Terra Rossa soil, dominated by the following main vegetation: *Rhamnus lycioides*, *Amygdalus communis*, the endemic *Salvia judaica* Boiss., *Lomelosia palaestina*. Besides, several noticeable species such as *Scorzonera phaeopappa* (Boiss.) Boiss., *Ophrys sphegodes* Mill., the two sub-endemic and very rare species of *Ornithogalum fuscescens* Boiss. & Gaill. and the endangered species of *Alkanna galilaea* Boiss., and the very rare species of *Salvia pinnata* L. *I. haynei* occurs in different places within Faqqua and Jalbun towns, but the survey did not cover the whole area (see discussion for more details). This site is under the main threat of urbanization and traditional agricultural practices, which may have an effect on the *I. haynei* population in the future.

Discussion

The flora of Palestine has not been studied well (Levin and Shmida 2007; Danin 2015; EQA 2021), especially for the endemic, threatened and rare plant species. These data are vital for building the database of any country, conservation and management purposes, and to be disseminated to the global scientific communities for conservation

Figure 10. Images for four of the described habitats of *I. atrofusca*; **A.** Hamam Al Malih – Tubas district; **B.** Wadi Firan – Tubas district; **C.** Khirbet Yarza I area – Tubas district; **D.** Khirbet Yarza III area – Tubas district.

actions. In this study, a comprehensive plant survey was conducted to evaluate the occurrence, distribution range, population size, and habitat of the three Royal Irises in Palestine, mainly in the north-central parts of the West Bank. Populations of *I. lortetii* were documented in nine different sites within Nablus district. Yasid area, which is the largest studied site, held the highest population of *I. lortetii*. Various clones were non-flowering clones, indicating that a high ratio of younger generations is being established. The documented habitats and vegetation of all studied sites reveal that these areas possess high richness in species and the provision of ecosystem services. The highest recorded elevation of *I. lortetii*'s population was at 769 *mamsl* within Mount Nablus (Mount Ebal ~938 *mamsl*) in the central part of Nablus district, whereas the lowest recorded population was at the Al Bathan II site at an elevation of 176 *mamsl*. Aqraba II site is a Mediterranean zone, but it is penetrated by the Irano-Turaninan

Figure 11. Images for the described habitats of *I. haynei*; **A.** Raba I area (Man-made Forest) – Jenin district; **B.** Faqqua site – Jenin district; **C.** Raba II area – Jenin district.

and Saharo-Arabian zones since the two the sub-endemic *Trichodesma boissieri* and the endemic *Matthiola aspera* (Feinbrun-Dothan 1978; Danin 2015) were observed in several locations. These two species are typically spread in the eastern slopes of Nablus Desert and up north to the Wadi Talkid area (North Central part of the lower Jordan Valley, Dead Sea area) (Feinbrun-Dothan 1978; Danin 2015; Horvitz 2016).

Furthermore, Aqraba II site has the highest percentage of flower-borne clones of *I. lortetii* in a relatively small area of 68 dunams (0.068 km^2), probably due to minimal anthropogenic activities. This revealed that the site has the highest population density of *I. lortetii*'s, and the majority of the recorded clones are adults (flowering clones). Moreover, the site is under threat of being invaded by the American invasive plant species (Feinbrun-Dothan 1978; Danin 2015), mainly *Erigeron bonariensis* L. and *Erigeron canadensis* L. (Asteraceae) where several populations were recorded, these species are spreading rapidly (Feinbrun-Dothan 1978). Finally, several sites within the studied area were not surveyed comprehensively (only several clones were recorded such as in Al Jabal Al-Kabir (Al Bathan), because these sites are either close to Israeli settlements, within an Israeli military base, or being an Israeli army training zone. Therefore, a comprehensive survey should be conducted to evaluate their potential for having populations of *I. lortetii*. The typical *I. lortetii's* scape is as long as the leaves, where its falls have purple or reddish veins and minute dots (Feinbrun-Dothan 1986). Our observations of the variability in flowers' coloration indicate that the flowers of *I. lortetii* have more color variations than documented in the literature (Feinbrun-Dothan 1986). The difference in coloration pattern is contrasting between Mount Ebal and the northern region of Yasid. The unique color diversity of the *I. lortetii*'s localities of Mount Ebal (*Nablus*) is distinguished by the creamy ground color of the standard petals and the purple-to-brown color of their veins. Whereas the northern sites such as Yasid localities, where Iris' standard petals have pale lilac veins on a nearly pale misty rose ground as well as having various flower colorations such heliotrope purple standards with brown falls and lilac sepals with dark purple veins and brown falls with very dark brown spots and black background beneath the beard (Table 2 and Fig. 4J, L).

Based on Danin 2015 and Horvitz 2016, who reported the occurrence of a small population of *I. lortetii* near the Israeli settlement of Itamar in the central West Bank, this area was not covered in our study due to safety concerns.

In addition, the Itamar settlement is located in the middle area between Mount Ebal and Usarin, which might indicate that *I. lortetii* localities extend along this area, even in very small numbers. Finally, *I. lortetii* is limited in its distribution due to self-incompatible and obligatory pollination by male bees which causes limited dispersal of a few tens of meters only and low genetic diversity as shown in other related *Iris* species (Segal et al. 2006; Shemesh et al. 2018). These issues highlight the importance of active conservation through, at least, In-situ and Ex-situ conservation.

New populations of *I. atrofusca* were documented for the first time in areas that held one of the highest numbers of clones ever recorded. These sites are in the eastern parts of Tubas district, within transition zone of the Mediterranean and Irano-Turanian. The Khirbet Yarza area (sites of Yarza I, II, II, and IV) holds the highest number followed by Khirbet Ar-Ras Al Ahmar and Hamam Al Maleh. However, the number of *I. atrofusca* clones is expected to be higher, since mainly mature and flowering clones (patch of clones) were counted. Interestingly, two clones at Wadi Firan (Table 3), are located at an altitude of -209 *mbmsl*, which is the lowest elevation known of *I. atrofusca* ever recorded within its natural distribution range. Moreover, the Al Taybeh site (Taybeh I and II), held two populations of *I. atrofusca* with a total number of 218 clones and 697 flowers. The documented inflorescence-coloration variation of *I. atrofusca* ranges from yellow-orange fall sepals, with maximum purple standard petals tinted orange at their base with dark brown background beneath an orangish beard (Fig. 12A), to white standards with light pastel magenta falls, whereas on the same clone, it has dark brown-purple to nearly black fall petals, with standards that are erectly incurved with dark purple-brown to dark purple radiating veins and dense dots (Fig. 12B).

It is observed that the further north the distribution area of I. atrofusca extends, the more variability there is in its perianth color, with its flowers becoming more purple

Figure 12. Extreme inflorescence-coloration variation of *I. atrofusca*. **A.** Inflorescence-coloration variation for the yellow-orange fall sepals, with maximum purple standard petals tinted orange at their base with dark brown background beneath an orangish beard. **B.** Inflorescence- coloration variation for the same clone which carries both white standards with light pastel magenta falls, and the dark brown-purple to nearly black fall petals and dark purple- brown standard petals.

Figure 13. A, B. Shows the increasing intensity of purple coloration in *I. atrofusca* flowers towards the northern part of their distribution, with standards displaying dark purple veins on a paler background, a trait also observed in *I. haynei*.

and the standards showing dark purple veins on a paler background, a feature also found in *I. haynei* (Fig. 13A, B).

This feature might be an introgression hybridization between *I. atrofusca* from *I. haynei* (Feinbrun-Dothan 1986). However, the main characteristic shape of the *I. atrofusca*'s flowers is maintained throughout its distribution range; the flower is as long as broad, not longer than broad as in its peer *I. haynei* (Feinbrun-Dothan 1986; Sapir and Shmida 2002).

Three new sites for *I. haynei* were discovered in Raba area (Raba I, II, and III), which is in the northeastern part of Jenin district, about 12 km in a west-south direction of the known site of *I. haynei*, the Faqqua-Jalbon area. The Raba area was found to hold a high population density of *I. haynei*, which was unknown to the scientific communities at the national and international levels (Feinbrun-Dothan 1986; Danin 2015; Horvitz 2016). The area is home to about 10729 clones of *I. haynei*, which exceeded the population size, recorded in previous studies within the Faqqua-Jalbun area by several folds. However, these sites are under major threats, Raba II & III sites are typical rocky batha hills that are under severe pressure of overgrazing, which indirectly affects the population of *I. haynei*.

Many *I. haynei* populations were found either devastated by the sheep and goat herds, flowers were picked and collected, clones were uprooted of the rhizomes. The ripe fruit capsules were either bisected or destroyed. The Raba I site is located within a man-made coniferous woodland, where *I. haynei* populations face various threats, such as a shortage of available sunlight due to the dense coniferous trees. Their rhizomes are buried under the needle-like leaves of the coniferous trees, which are also known to alter the soil pH by increasing its acidity. Additionally, there is interspecific competition between the pine trees and other natural vegetation, including *I. haynei*, for resources. All these factors indicate an unhealthy population of *I. haynei*, which might explain its decreased number of flowers (4201) despite the highest reported number of clones (7276, Table 5), in contrast to Raba II site, where a lower number of clones (3281) were documented with a higher number of flowers (5057). Moreover, the highest elevation recorded for *I. haynei* populations was within Raba II site (647 *mamsl*) whereas the lowest elevation was within Faqqua area (334 *mamsl*). To our surprise, the *I. haynei* population of Faqqua site was already in the ripe fruit's capsules and the clones were already dry on the 28th of March (Fig. 14A, B), which is considered a very early stage in the life cycle of the *I. haynei*, where the ripe fruit's capsules are normally displayed by May.

In addition, various geophytes such as *Ophrys sphegodes* Mill (normally it starts blooming in the third week of March until the end of April) and *Anacamptis papilionacea* (L.) R.M.Bateman, Pridgeon & M.W.Chase (blooming period is mid of February until the first week of April) were already dry (Feinbrun-Dothan 1986; Danin 2015). This phenomenon could be an acclimation or adaptation process for the *I. haynei* and other geophytes to cope with the new environmental conditions due to climate change, where rain seasons are shorter, the mean temperature is increasing, and the summer season is becoming longer, which lead to early blooming (Zittis et al. 2022). Therefore, the field survey was terminated for the spring season of 2023 at the Faqqua-Jalbun area after the first visit on the 28th of March to be continued earlier in the next spring of 2024. It is quite unexpected to describe and document seven variations in the coloration of *I. haynei* inflorescences. Some of the extreme colors range from white standard petals tinted with a pale lemon-yellow hue, accompanied by lemon-yellow fall sepals, to white standard petals tinted with a pale amber hue, accompanied by light amber-orange fall sepals, and to very pale lilac standard petals with a pale rosy background and fall sepals moderately adorned with numerous lilac dots. The variance in color is due to a deficiency in anthocyanin which is reflected in the white tinted with- yellow flowers (Feinbrun-Dothan 1986; Fig. 8D, E).

Floral color has a key role as a visual signaling sign that paves the pathway of plant-pollinator interactions. A study that examined the evolution of flower colors in the genus Royal Irises in a micro-evolutionary framework

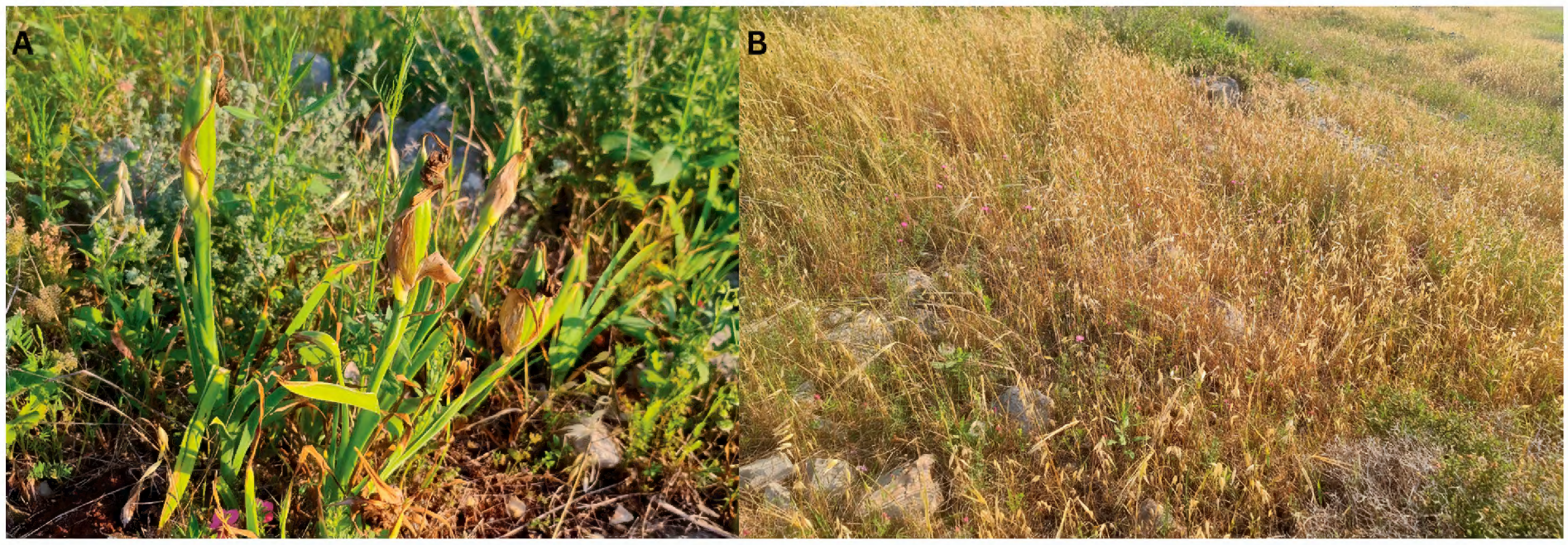

Figure 14. A, B. Within Faqqua- Jalbun site the *Iris haynei* ripe fruit's capsules and the clones had already been dried on the 28th of March, where this developmental stage (ripe fruit capsules) is typically seen within the month of May. This could be an acclimation process for *Iris haynei* and other geophytes to cope with the new environmental conditions.

revealed that the most recent ancestor of Royal Irises was monomorphic with purple flowers that had a crest and a spot on its fall (Roguz et al. 2020). The diversity in the detected flower coloration within the modern Royal Irises represents a trade-off between conflicting selection pressures (Souto-Vilarós et al. 2018; Roguz et al. 2020). More research is needed to determine whether these phenotypic traits (inflorescence-coloration variation) are due to abiotic or biotic selective agents, neutral processes without any selection, genotypic traits, or environmental pressure. The genetic and physiological mechanisms governing the variable flower coloration in the genus *Iris* remain to be elucidated. Further genetic analysis is needed to confirm the proposed introgressive hybridization between *I. haynei* and *I. atrofusca* (Feinbrun-Dothan, 1986). Additionally, genetic studies are required to clarify the taxonomic status of *I. lortetii* in the Nablus region, to determine whether it represents a distinct variant (*I. lortetii* var. *samaria*) or if it is consistent with the populations found in Upper Galilee and southern Lebanon.

Conclusion

All three Royal Irises are very narrow endemics (Feinbrun-Dothan 1986; Danin 2015) found within a limited area that does not exceed 355 km^2. The uncovering of new sites of the endemic and vulnerable *I. haynei* (12 km to the west-south of the only known site-Faqqua-Jalbun area) and the sub-endemic and vulnerable *I. atrofusca* with high recorded numbers of clones and flowers suggest that the distribution range and the population size of these two *Iris* species are higher than previously reported. In addition, the high numbers of clones and flowers of the sub-endemic and endangered *I. lortetii*, shows that its population size is larger than expected (Horvitz 2016; IUCN 2016). Therefore, the IUCN status of the three Royal Irises should be re-evaluated and updated accordingly.

In addition, it would be of interest to conduct a population genomic analysis on the known population across the distribution range of the *I. lortetii*, coupled with phylogenetic analyses and coalescent simulations, which could provide valuable information on the origin, demographic histories, and adaptation (diverge at candidate loci) to their distinctive habitats. Similar studies should be carried out on the populations of *I. haynei* and *I. atrofusca* along their distribution range to clarify any ambiguity regarding these species. These future genetic studies shall help in understanding the evolutionary processes over small spatial scales besides examining the variations among the species.

All recorded populations of the three Royal Irises are under different types of anthropogenic threats and the effects of climate change require determining their influence upon the life cycle of the Irises, which might lead to several consequences, including extinction. The lack of awareness among residents is an important factor to be taken into consideration, as a major threat to Royal Irises' populations in Palestine. Therefore, conservation actions, protection measures, and increasing public awareness is essential to sustain and conserve the populations of the threatened and endemic Royal Irises in Palestine.

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Competing interests

The author has declared that no competing interests exist.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author Anton Khalilieh (anton@naturepalestine.org).

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Supplementary material 1

Iris species

Authors: Johann Gabriel Gedeon, Anton Issa Khalilieh

Data type: xlsx

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